Effects of fitch odor (*Mustila eversmanni*) on behavior and breeding of root voles (*Microtus oeconomus*)

WANG Zhen-long, MA Wen-xiang
(*Department of Biology, Qufu Normal University, Qufu 273165, P.R. China*)

Abstract: From April to December in 1997 at Northwest Plateau Institute of Biology, Chinese Academy of Science, the effect of predation risk on social behavior, feeding, reproduction and sexual hormones of the root voles (*Microtus oeconomus*) was studied when the root voles (*Microtus oeconomus*) were exposed to the odor of fitch (*Mustila eversmanni*). The results showed that the root voles delayed breeding period and the frequency of the copulation significantly lowered ($P<0.01$). Comparing with the control group, the accumulated time of amicable behavior in females, and general activity of both sexes increased significantly ($P<0.01$). The frequencies of general activity ($P<0.01$), investigating activity (females: $P<0.05$, males: $P<0.01$), and self-grooming ($P<0.01$) of both sexes were less significantly. At the same time, the amount of energy intake ($P<0.05$) and body weight ($P<0.01$) of the root voles decreased significantly. The litter weight and ovary index of females decreased ($P<0.01$). For males the sperm count in epididymides also decreased significantly ($P<0.01$). The progesterone in the blood serum of root voles females increased significantly under fitch odor ($P<0.01$).

Key words: Fitch (*Mustela eversmanni*); Root voles (*Microtus oeconomus*); Behavior; Breeding
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Introduction

Predation has long been implicated as a major selective force in the evolution of several morphological and behavioral characteristics of animals (Lima *et al.* 1990). The effects of predator odor are frequently reported significantly on social behavior (Ylonen *et al.* 1989; Ylonen *et. al.*, 1992), foraging behavior (Batzli 1986; Lima *et al.* 1986), and its reproduction (Ylonen *et al.* 1989; Ylonen *et al.* 1992; Esa *et al.* 1995). However, there are only few studies on predation risk altering reproduction (Esa *et al.* 1995), even less on mammals. The purpose of this study is to determine experimentally how the indirect cues of mustelids affect the social behavior, feeding, reproduction and sexual hormones of root voles (*Microtus oeconomus*). Further, we also discuss the possible ultimate and proximate causes after delayed breeding period.

Materials and methods

Study sites and materials

We carried out the study at Northwest Plateau Institute of Biology, Chinese Academy of Science, from April to December in 1997. Root voles and fitches were captured for experiment in the site of Haibei Alphine Meadow Ecosystem Research Station of Chinese Academy of Science, which located in northwest Qinghai-Tibet Plateau (37°29′-37°45′N, 101°12′-101°23′E). And they were housed individually in the standard breeding cages (29 cm×19 cm×13 cm). Water, Purina rabbit chow, and carrot were provided to the root voles in the experiment. The females were not pregnant at the beginning of study. All the root voles used in experiments were (non-overwintered) mature adults, and the males had scrotal testes. We randomly chose voles for the experiment. There was a little difference between the control weight (W_{co}) and experimental weight (W_{ex}) for voles at the beginning of the experiments (females: W_{ex}=(27.4±3.0) g, W_{co}=(28.5±2.9) g; *t*-test, t= -0.90, d*f*=22, P=0.380; males: W_{ex}=(45.7±5.6) g, W_{co}=(45.2±6.8) g; *t*-test, t=0.25, d*f*=29, P=0.806). Fitches (*Mustila eversmanni*) caught from the wild were used to obtain mustelids odor.

Study arenas and methods

We carried out 18-day experiment in two main arenas (first three days) and in breeding cages (15 days thereafter) indoors. The arenas of 100 cm×40 cm×40 cm were made of plexiglas. Every arena was divided longitudinally with a plexiglas wall into two even-sized compartments connected with a plexiglas tube. Prior to introducing the root voles pairs into the main arenas, the sawdust was daily sprayed in the experimental arenas with filtered dilution of urine and odor of fitch. Of the two compartments of the arenas, only one was sprayed. This enabled the voles to choose a less contaminated part of the arena. The control arenas were sprayed daily with distilled water. We used predator odor instead of a real predator to avoid any superfluous stress to the experimental animals. We had no null-treatment with a novel odor because previous studies had shown that they had no effect on the behavior of *Mi-*

Biography: WANG Zhen-long (1971-), male, lecture in Department of Biology, Qufu Normal University, Qufu 273165, P.R. China
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crotus species (Calder *et al.* 1991).

The effects of fitch odor on the behavior of voles were observed in behavior arenas. Each trial was carried out in the afternoon, and lasted for 40 minute. Before the start of the trials, the voles were kept in separate tubes on opposite sides of the behavioral arena for 1-2 minutes and then released simultaneously. The activates were recorded by counting the frequencies and measuring total time of all variables (Esa *et al.* 1995), such as general activate (active movement or staying in one place), amicable interactions, aggressive behavior (mainly threats and attacks), self-grooming (grooming body or pudenda of itself), Investigating behavior (approaching, sniffing body or pudenda of opposite sex), and copulatory behavior (mounts and intromissions preceding ejaculatory). The copulatory behavior was recorded as the times of each pair practicing copulatory attempts.

The 34 (17 experimental individuals and 17 control individuals) individuals with female-male pairs of voles were chosen. In the first day of the study, the mating and foraging (the amount of energy intake) behaviors were observed. Then root vole pairs were transferred to the breeding cages where the breeding experiments continued. Water, Purina rabbit chow, and carrot were provided enough for the root voles in the whole experiment. On the 18th day, all root voles were killed and weighted. The reproductive stage, weight of ovaries and the number of embryos were measured in females, as well as the number of spermatozoa, weight of epididymis in males.

After the behavior observation period, each pair of root voles was moved from the trial arenas to breeding cages for foraging trail. We recorded the amount of root voles eating up Purina rabbits chow in 24 h. Then the root voles were moved to the breeding cages for 15-day breeding trial. They were situated indoors under light/dark (12:12h) photoperiod and (20±1)℃ temperature, and were not disturbed except for feeding and daily spraying with odor or distilled water.

We used ANCOVA to analyze the difference between the initial weight and the final weight of the root voles, and used one-way ANOVA (one-way analysis of variance), Student's *t*, and Pearson's x^2 with Yates's test to analysis the difference among other variables. The behavior variance was analyzed by SPSS10.0. Variable values are given as mean±SE. All the tests are two-tailed.

Results

Reproduction and social behavior

Two of 17 females in the experimental group and 16 of 17 females control group were observed for mating behavior respectively (Pearson's x^2 with Yates's = 19.95, P=0.000). The greater proportion of mating behavior of root voles in control group (94%) proved that the root voles were in reproductive condition. 14 of 17 females in the experimental group and 5 of 17 females in control group postponed breeding, respectively (Pearson's x^2 with Yates's = 7.64, P=0.006). On average, 1.50 (SE=0.50) ejaculatory series were observed in the experimental group, while, 3.71 (SE=0.86) in the control group.

During the behavior observation period, the frequencies of general activity (P<0.01), investigating activity (females: P<0.05, males: P<0.01), and self-grooming (P<0.01) of voles were less significantly in experimental group than in the control group (Table 1). Females tended to be more aggressive and amicable activity frequently, and males tended to be more amicable and less aggressive activity frequently under the fitch odor.

Table 1. Mean frequency (M±SE) of root vole behaviors under fitch odor in Wilcoxon unpaired test

Behavioral variance	Females			Males		
	Fitch odor	Control	*P*	Fitch odor	Control	*P*
Amicable behavior	10.75±2.45 (13) *	8.00±1.21 (13)	0.794	9.73±2.86 (11)	7.91±1.20 (11)	0.741
Aggressive behavior	45.07±5.58 (14)	44.50±4.80 (14)	0.963	0.21±0.15 (14)	1.50±0.72 (14)	0.146
General activity	54.00±5.30 (15)	81.67±3.10 (15)	0.000	53.62±4.76 (14)	86.50±2.40 (14)	0.000
Investigating activity	10.92±2.55 (13)	18.31±1.58 (13)	0.050	49.45±5.48 (11)	77.55±2.90 (11)	0.002
Self-grooming	2.06±0.64 (16)	11.63±0.78 (16)	0.000	2.69±0.67 (16)	14.81±1.13 (16)	0.000

Note: * --Values in parenthesis are sample size.

Results of Wilcoxon unpaired test for the accumulated time of root voles behaviors under fitch odor are summarized in Table 2. Under fitch odor, the accumulated time of amicable behavior of female (P<0.01) and general activity of both sexes increased significantly (P<0.01), females tended to be less aggressive under fitch odor.

Table 2. Mean accumulated time (M±SE) of root vole behaviors under fitch odor in Wilcoxon unpaired test

Behavioral variance	Females			Males		
	Fitch odor	Control	*P*	Fitch odor	Control	*P*
Amicable behavior	40.70±12.79 (10) *	18.69±2.24 (16)	0.000	59.40±20.07 (10)	30.13±3.16 (15)	0.845
Aggressive behavior	22.77±4.38 (13)	41.85±5.27 (17)	0.032	0.19±0.14 (16)	2.87±1.48 (17)	0.213
General activity	2 256.29±20.88 (14)	2 083.25±31.29 (17)	0.000	2 042.43±44.91 (14)	1 752.58±46.50 (16)	0.000
Investigating activity	15.00±4.82 (11)	36.16±4.04 (16)	0.003	204.78±25.56 (9)	154.07±15.90 (14)	0.192
Self-grooming	3.93±1.70 (14)	78.92±6.73 (13)	0.000	16.15±5.83 (13)	143.25±12.66 (16)	0.000

Notes: * --Values in parenthesis are sample size; Mean accumulated time (M±SE) is computed in terms of second.

Foraging and weight

The effects of fitch odor on energy intake amount of root voles are summarized in Table 3. Under the fitch odor, in two treatments (15 minutes and 24 hours), the amount of energy intake in both sexes decreased significantly (P<0.05).

Table 3. The effects of fitch odor on intake energy amount of root voles

Time	Females			Males		
	Fitch odor	Control	t-test	Fitch odor	Control	t-test
15 min	5.45±1.17 (12) *	10.06±1.85 (7)	0.039	7.90±1.25 (14)	12.89±1.40 (7)	0.030
24 h	49.06±8.01 (10)	81.49±9.88 (7)	0.038	94.38±8.35 (14)	123.88±5.47 (7)	0.029

Notes: *--Values in parenthesis are sample size; Intake energy amount is computed in terms of joule.

The weight of females and males in the experimental group differed significantly with those in control group at the end of the experiment (Table 4). Females in experimental group lost weight under predator odor but gained weight in the control group (in experimental group: mean weight change d=-3.1, t=-4.25, df=16, P=0.013, in control group: d=5.1, t=4.81, df=15, P=0.000 in Fig. 1). A significant decrease in the weight of the males was found in the experimental group but not in the control group (in experimental group: mean weight change in grams d=-2.2, t=-3.38, df=16, P=0.017, in control group: d=-0.8, t=-0.62, df=15, P=0.613 in Fig. 2). Weight loss under predator odor was due to decrease in foraging. During the 24-hour observation period, in the experimental group root voles ate significantly less than those in the control group (females: W_{ex}=(3.31±0.28) g, W_{co}=(5.31±0.53) g, t-test, t=2.63, df=20, P<0.01; males: W_{ex}=(5.71±0.74) g, W_{co}=(6.71±0.75) g, t-test, t=2.18, df=20, P<0.05).

Table 4. Difference between final and initial weight of the females and males with ACONOVA (M_S) table

Source of variation	df	M_S	F	P
Females				
Initial weight/g	1	278.314	105.484	0.000
Treatment	1	534.631	202.631	0.000
Error	23	2.638		
Males				
Initial weight/g	1	712.779	71.566	0.000
Treatment	1	195.215	19.600	0.002
Error	23	9.960		

Reproductive parameter and sexual hormone

Results of analysis on reproduction parameter of root voles were summarized in Table 5. The sperm count (N_{ex}=8.87±0.96×10^8, N_{co}=16.70±1.42×10^8, one-way ANOVA, F=21.30, df=1,30, P<0.001) of males, litter weight (W_{ex}=12.66±1.12 mg, W_{co}=240.44±19.35 mg, one-way ANOVA, F=23.05, df=1,33, P<0.001) and ovary index (O_{ex}=0.24±0.01, O_{co}=0.37±0.03, one-way ANOVA, F=14.51, df=1, 31, P<0.001) of females decreased significantly under fitch odor. But the litter size, epididymis index, and testis index did not differ between those in the experimental and control group. The progesterone in the blood serum of females increased significantly under fitch odor (F=18.26, df=1,29, P<0.01), but there was no difference in testosterone in the blood serum of males between those in experimental and control group (F=0.01, df=1,22, P>0.05).

Discussion

The root voles under small mustelid predation risk delayed their breeding period. These results agree with the theoretical considerations by Stearns (1976), Wasser and Barash (1983) on optimal breeding tactics in a temporally heterogeneous environment. In the environmental group with high predation risk, decreased breeding and foraging behavior possibilities may form the basis for the ultimate explanation for delayed breeding, that is, changing in mating behavior and poor nutritional condition leading to infertility (Ronkainen *et al.* 1994; Esa *et al.* 1995).

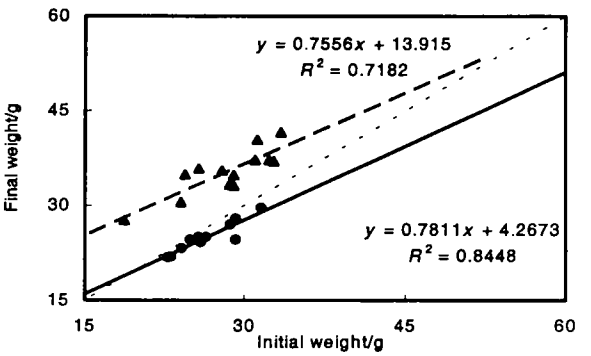

Fig. 1 Weight differences of the female root voles in the control and the experimental group

-▲- Control group; -•- Experimental group

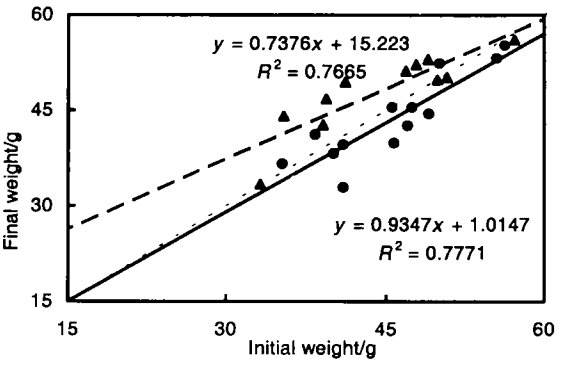

Fig. 2 Weight differences of the male root voles in the control and the experimental group

-▲- Control group; -•- Experimental group

The fitch odor strongly affected the mating behavior of the voles. The copulatory behavior was observed in 16 pairs of the control group but only 2 pairs of the experimental group. The frequencies of general activity, investigating activity, and self-grooming of voles were less significantly in experimental group than in the control group, and females tended to be more aggressive and amicable activity frequently, and males tended to be more amicable and less aggressive activity frequently under the fitch odor (Table 1). The explanation for this is obvious as there were no copulatory attempts. The accumulated time of amicable behavior of female and general activity of both sexes increased significantly, females tended to be less aggressive under fitch odor (Table 2). Ronkainen and Ylonen (1994) suggested that females actively avoid copulations and decide not to breed when exposed to predators. Our results supported this assertion (Table 2).

The foraging activity of the root voles decreased under predation risk as reported early for other rodents (Esa *et al.* 1995). The voles exposed to mustelid odor did lose weight during the experiment (Ylonen *et al.* 1994; Esa *et al.* 1995). The decrease in foraging activity, possibly leading to malnutrition and infertility of individuals, could be the cause for the observed breeding suppression (Batzli 1986). Esa and Ylonen (1995) suggested that hormone changes could be caused by predation risk.

Table 5. Reproductive parameter (M±SE) with one-way ANOVAs for root voles under fitch odor

Reproductive parameter	Fitch odor	Control	*F*	df	*P*
The sperm count ($\times 10^8$)	8.87±0.96 (16) *	16.70±1.42 (15)	21.30	1, 30	0.000
Litter sizes	4.46±0.35 (13)	4.27±0.21 (15)	0.24	1, 27	0.625
Litter weight/mg	12.66±1.12 (17)	240.44±19.35 (17)	23.05	1, 33	0.000
Testis index**	11.18±0.45 (17)	11.28±0.70 (16)	0.25	1, 33	0.628
Epididymis index**	1.60±0.10 (16)	1.95±0.17 (16)	3.04	1, 31	0.091
Ovary index**	0.24±0.01 (16)	0.37±0.03 (16)	14.51	1, 31	0.000

Notes: *--Sample sizes are given in parentheses; **--Organ index = organ weight (mg)/body weight (g).

According to Stearns (1976), a strategy to delayed reproduction could be profitable, if breeding suppression can increase the probability of an individual's surviving to the next breeding opportunity, or if the value of the young born during the current breeding season is low.

To conclude, our results indicate that the proximate mechanism causing the delayed breeding at least partly based on changes in the sexual behavior and foraging of root voles, we can exclude the possibility that sexual hormone changes of females may affect the reproduction of root voles.

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